1% of the activity exhibited by wild-type pneumolysin. Support for additional claims 33 and 34 is found in the specification at, inter alia, page 54, Table 5A which describes various polypeptides and their residue substitutions.

In response to the Examiner's comments in Section 3 entitled "Election," applicants appreciate the Examiner's consideration of the possible inclusion of the subject matter of claims 27-29 at a future date.

RESPONSE TO SPECIFICATION INFORMALITIES

The Examiner contends that Figure 14 is confusing in the designations: PCYD mutant A and PCYD mutant B. *See* Section 8(a). The Examiner's attention is directed to the legend of Figure 14 on page 10 of the application. Here it is clearly stated that PCYD mutant A is pNV207 and PCYD mutant B is PNV103. The amino acid substitutions they carry can be found throughout the application, for example, in Table 5A on page 54.

Drawings 1-4 and the corresponding description in the specification are objected to by the Examiner for improper labeling of the subparts. Applicants have amended the specification to comply with the Examiner's recommendations. Formal drawings complying with the Examiner's recommendations will be submitted in due course.

RESPONSE TO REJECTION UNDER 35 U.S.C. § 112

Claims 1-3 and 22-26 stand rejected under 35 U.S.C. § 112, first paragraph, because the Examiner contends that the specification does not reasonably provide

enablement for a modified or attenuated pneumolysin polypeptide obtained by a mutation at any one random position, or more than one position as claimed in a broad sense. Applicants respectfully disagree with this rejection.

The Examiner's rejection relies on a Wand's analysis and a contention that applicants' disclosure itself "suggests that the entire scope of the claims is not enabled." Although the Examiner states that "the relative skill of those in this art is high," the Examiner contends that the claims are too broad and protein folding and attenuation of hemolytic activity are unpredictable. The Examiner relies on applicants' specification to conjecture that "with a substitution at a single amino acid position (let alone combination of substitutions), the refoldability of the resultant single mutant polypeptide is an unpredictable event." The Examiner also cites several publications in connection with her contentions that "attenuation of the hemolytic activity of a wild-type pneumolysin by any random mutation is unpredictable."

Regarding the Wands factors, applicants point out that they "are illustrative, not mandatory. What is relevant depends on the facts . . . ." *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd.*, 927 F2d 1200, 1213 (Fed Cir. 1991). The Examiner's analysis must consider all the evidence, and any conclusion of non-enablement must be based on the evidence as a whole. *In re Wands*, 858 F.2d 731, 737 and 740.

Although the Examiner has acknowledged that "the relative skill of those in this art is high," she merely contends that the claims are broad and that protein folding and hemolytic attenuation are unpredictable. As amended, all of the claims require at least

one amino acid substitution in the first 257 amino acids of the polypeptide. Polypeptides within the scope of the claims may be obtained by random mutation of the nucleic acid molecule corresponding to amino acids 1-257 of SEQ ID No: 3 and expressing the mutated nucleic acid in a host cell. The expressed protein is assayed for hemolytic activity, and those polypeptides which have attenuated herolyte activity, substantially similar molecular weight as native wild-type pneumolysin and are refoldable are identified and selected. Although the potential number of different polypeptides is large, applicants provide nine working examples, some with single amino acid substitutions, some with multiple amino acid substitutions.

The state of the art and amount of direction provided by applicants' specification are such that one of ordinary skill in the art can practice applicants claimed invention without undue experimentation. For example, of approximately 10,000 colonies, 400 were randomly selected for evaluation according to the following criteria: (1) low hemolytic activity, (2) substantially full-length, (3) partially soluble, and (4) monomeric and refoldable. Nine different polypeptides within the scope of applicants' claims were obtained; five with multiple mutations and four additional polypeptides with single mutations which were derived from the original five multiply mutated polypeptides.

The Examiner has not provided any evidence that a skilled artisan could not repeat applicants' process and obtain additional polypeptides that would fall within the scope of the amended claims. The Examiner relies on instances of an amino acid substitution either affecting the refoldability of a pneumolysin polypeptide or not

effecting attenuation of hemolytic activity. However, the focus of analysis should not only be on the instances of mutations resulting in pneumolysin polypeptides not falling within the scope of applicants' claims. Indeed, not all mutations result in the production of pneumolysin polypeptide having desired characteristics; only 200 of 400 randomly mutated clones were selected based on a hemolytic assay. Rather, the focus of analysis should properly be concentrated on whether one skilled in the art, when practicing applicants' invention, will predictably create pneumolysin polypeptides that fall within the scope of the present claims. An analysis of this question in light of the Wands factors leads to the answer that, applicants' invention predictably produces the claimed pneumolysin polypeptides possessing the desired characteristics.

The Examiner makes additional contentions that "there is no evidentiary support in the specification that a mutant with more than one mutation, if constructed, would be hemolytically attenuated and immunogenically functional . . ." The Examiner is mistaken as to the lack of evidence of multiple mutations effecting attenuated hemolytic activity. Evidence for pneumolysin polypeptides having multiple mutations and having attenuated hemolytic activity is found in Table 4. Polypeptides pNVJ22, pNVJ20, pNVJ1, pNVJ45 and pNVJ56 are all polypeptides that fall within the scope of the pending claims, and have multiple mutations. Thus, the specification provides adequate enablement for the modified pneumolysin polypeptides of the amended claims. Although the potential number of polypeptides covered by the claim is large, this factor is not dispositive when viewed in light of the remaining Wands factors. Applicants

describe a method that does not depend on predicting whether a particular mutation or set of mutations will attenuate hemolytic activity and allow for protein folding. Rather applicants' method produces many mutants of which, according to the method, it is predicable that pneumolysin polypeptides with attenuated hemolytic activity and proper folding will be obtained without undue experimentation. Thus, the proper question is whether applicants' invention will predictably create pneumolysin polypeptides that fall within the present claims, and not whether pneumolysin polypeptides that do not fall within the present claims may also be created. The specification provides substantial direction as well as working examples.

Claim 3, which limits the level of hemolytic activity, is patentable under the analysis discussed above.

Claims 22-26 are directed to the modified pneumolysin polypeptide of claim 2 conjugated to polysaccharide. The Examiner contends that "modifications of a native polypeptide at multiple positions can potentially alter its conformational, antigenic and immunogenic integrity, and the use of such an excessively modified polypeptide as a protein carrier would potentially result in a non-effective conjugate vaccine." The Examiner further contends that "[t]here is no evidence within the instant disclosure that such a modified pneumolysin would function effectively either in a conjugate or in a non-conjugate form."

Applicants describe the production of polysaccharide/modified pneumolysin conjugates of pNV103, pNV207 and pNV111 which are all single mutation polypeptides.

Table 11 shows these conjugates are immunogenic. The Examiner has not provided any evidence that conjugates could not be made with modified pneumolysin polypeptides containing multiple mutations. The Examiner merely contends that "[t]here is no evidence within the instant disclosure that such modified pneumolysin would function effectively [ ] in a conjugate [ ]." However, with respect to the modified pneumolysin functioning as an effective carrier to convert a thymus independent response to one which is thymus dependent, there is no reason to believe that pneumolysin with multiple mutations and which is substantially the same size as wild-type and is refolded would not function similar to both wild-type and to modified pneumolysin with one modification.

The Examiner's arguments conclude with the contention that "in light of the demonstrated unpredictability of obtaining an attenuated pneumolysin by any random mutation and in light of Applicants' own evidence showing that refoldable property even of a single mutant of pneumolysin is an unpredictable event, and the lack of or inadequate specific guidance and direction provided in the instant disclosure as to how to reproductively obtain attenuated refoldable pneumolysins by random mutation(s) anywhere on the nucleic acid molecule of wild-type pneumolysins, or at any position in a region comprising amino acid residues 1 to 257 of type 14 pneumolysin, undue experimentation would have been required by one of ordinary skill in the art at the time of the effective filing date of the instant application to reproducibly practice the invention as claimed."

Contrary to the Examiner's contentions, applicants have demonstrated the ability to obtain useful polypeptides with a sufficiently high level of probability. In fact, applicants have demonstrated that of 400 clones examined, more than 200 had attenuated activity (> 50%). Of these, 200 were chosen and examined for full-length expression. Fifty-eight, or 29% (14.5% overall), were found that expressed modified pneumolysin with about the same molecular weight as native pneumolysin. Of these fifty-eight, five clones (8.6% or 1.25% overall) were found to express refoldable pneumolysin polypeptide which was expressed in high yield, and four additional clones were derived from these for a total of 9 clones. Thus, it is clear that one skilled in the art can obtain mutated pneumolysin with attenuated hemolytic activity according to applicants' invention.

Applicants' own evidence also demonstrates that obtaining a refoldable protein is a predictable event according to the invention. Fifty-eight clones which exhibited attenuated hemolytic activity and were of about full-length were examined for protein expression in the soluble fraction and for the ability of the expressed proteins to be denatured and refolded. Of the clones that passed all these tests, five (8.6% or 1.25% overall) were chosen which exhibited very high levels of expression. The five clones (pNVJ1, pNVJ20, pNVJ22, pNVJ45 and pNVJ56) which exhibited attenuated hemolytic activity, full-length and refoldability all contained multiple mutations. See Example 5. Based on the amino acid substitutions of these five clones, four additional single mutation pneumolysin polypeptides (pNV103, pNV207, pNV111 and pNV211) were

obtained by site directed mutagenesis. Thus, by following applicants' teachings, one of skill in the art would predict that other mutants within the scope of applicants' claims would be obtained in addition to those described above. Thus, the amount of experimentation necessary to discover new and useful strains is not undue since the process of random mutation and screening for hemolytic activity, protein size, and protein refoldability could be accomplished by routine and well-known methods.

The Examiner contends that applicants' discovery of seven mutants that were not refoldable (pNV21, pNV46, pNV22, pNV38, pNV40, and pNV20) is evidence that "the refoldability of the resultant single mutant polypeptide is an unpredictable event." However, when properly considered in an analysis similar to that of Wands, the exact opposite conclusion is reached. Of the fifty-eight clones that were of about full-length and exhibited attenuated hemolytic activity, five were found that were refoldable and expressed in high yields. Assuming that the remaining fifty-three clones were refoldable but not expressed in high yields (these are still within the scope of the claims), then 14.5% of the 400 clones originally examined fall within the scope of the claims. Even assuming that none of the remaining fifty-three clones were refoldable, the five found that were refoldable and expressed in high yields represents a 1.25% success rate. The Examiner has not provided any evidence that repeating applicants' procedure would not produce at least a 1.25% success rate. Even in Wands, applicant only had 2.8% probability of obtaining useful clones. In addition, among the claims which were appealed in Wands and which the court found to be enabled, were those pertaining to

chemically modified IgM antibodies immunoreactive to HbsAg. See, U.S. Patent 4,879,219 issued from Wands et al. application 188,735. Thus, it is clear that the methods and compositions covered by applicants' claims are sufficiently reproducible to enable one skilled in the art to obtain other useful polypeptides. Therefore, reconsideration and withdrawal of the Section 112, first paragraph, rejection is respectfully requested.

Claims 4-7 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which is most nearly connected, to make and/or use the invention. In particular, claims 4-6 are viewed as encompassing a modified pneumolysin polypeptide comprising at least one or a combination of substitutions. However, the Examiner contends that "no evidence exists within the instant specification that such modified pneumolysins, if produced, would be hemolytically attenuated and properly refolded as recited in base claim 2." Similarly, with respect to claim 7 which describes a modified pneumolysin with five amino acid substitutions, the Examiner contends that "no evidence is of record that establishes its refoldability." Further, the Examiner contends that "[t]here is no showing within the instant specification that pNVJ1, carrying multiple amino acid substitutions, is operable or functional, and that it retains the conformational assembly required for its immunogenic functions, and that it brings about the effects that are desired for." Applicants respectfully disagree with the Examiner's rejection.

As described above, Example 5 describes a method for selecting modified pneumolysin. The example describes the selection of clones based on (1) attenuated hemolytic activity, (2) expression of about full-length polypeptide, (3) expression of the polypeptide in the soluble fractions, and (4) refoldability. Of the clones that passed these evaluations (including refoldability, *see* page 51, line 21 through page 52, line 20) five clones with high yields of mononumeric modified polypeptides were selected. These clones which are pNVJ1, pNVJ20, pNVJ22, pNVJ45 and pNVJ56 all have multiple mutations and are refoldable.

It is unclear as to what the Examiner means by the contention that there is no showing that pNVJ1 is "operable or functional." A main goal of applicants' invention is to produce a non-functional pneumolysin, i.e., pneumolysin without hemolytic activity. In fact, this limitation is in all of the claims.

The Examiner's contention that "[t]here is no showing ... that pNVJ1 ... retains the conformational assembly required for its immunogenic functions ..." is incorrect. According to Example 5, modified pneumolysins are screened for refoldability based on their elution profile on a DEAE-Sepharose-FF column following refolding after denaturation in 3M urea. The selection of pNVJ1 for further analysis is consistent with a conclusion that this modified pneumolysin is properly folded and immunogenic.

Therefore, in light of the arguments presented above, applicants respectfully request reconsideration and withdrawal of the section 112, first paragraph, rejection.

Claims 1-6 and 22-26 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. None of the amendments to form are meant to limit the scope of the claimed invention.

(a) Claims 1 and 2 have been amended to include the recitation "SEQ ID NO: 3."

(b) Claim 1 has been amended to recite the various terms in singular form.

(c) Claim 1 has been amended to delete the recitation of "substantially" with respect to "similar."

(d) The term "properly refolded" in claim 2 is clear and distinct when viewed in light of the specification. For example, the specification describes that attenuated hemolytic activity can be the result of a truly inactive, properly refolded polypeptide or the result of simply being improperly refolded.

> Negative activity results imply either an inactive refolded polypeptide or an improperly refolded polypeptide. To distinguish between these two conditions, a second screening process can be used. Activity-negative clones are denatured and refolded before loading onto an ion-exchange chromatography column. The mutants which have an elution pattern similar to wild-type pneumolysin can be further analyzed by gel-filtration chromatography and mononumeric species with a Stokes radius similar to wild-type pneumolysin are selected.

See page 23, lines 5-14

> The properly refolded pneumolysin mutant should elute as a single peak between 13 and 20% Buffer B (25 mM Tris. HCl, 1 M NaCl, pH 8.0) similarly to what is observed for the wild-type. The protein peak is further analyzed by HPLC on a Superose 12 column and both elution time,

> aggregate/monomer ratio, and hemolytic activity are evaluated (*see* Table 4). The selected mutant(s) should present a single mononumeric species with a Stokes radius comparable to the wild-type.

See page 52, lines 5-13. Thus, properly refolded pneumolysin polypeptides are those that are refolded, are monomeric and have a similar elution profile to that observed for wild-type pneumolysin. However, in the interest of furthering prosecution, applicants have deleted the phrase "properly refolded" and substituted that phrase with "refoldable."

(e) "Polysaccharide" in claims 22 and 25 is a generic term used to describe a molecule comprising multiple monosaccharide units. The polysaccharide can be from any source, synthetic, mammalian, microbial, plant origin, etc. The only requirement in these claims is that antibodies elicited against the polysaccharide must also react with at least one bacterially derived polysaccharide. Ideally, the antibodies would also react with the bacterium from which the bacterial polysaccharide was obtained.

(f) Claims 23 and 26 have been amended to recite "bacterium" rather than "bacteria."

(g) Claim 23 has been amended to recite "meningococcus" rather than "meningococcal."

(h) Claim 24 has been amended to read "A vaccine comprising a pneumolysin polypeptide of claim 2...."

(i) Claim 26 has been amended to recite the conjunction "and" rather than "or."

(j) Claim 26 has been amended to recite "wherein the polysaccharide is a bacterial polysaccharide and is derived from a bacterium . . . ."

RESPONSE TO REJECTIONS UNDER 35 U.S.C. § 102

Claim 1 stands rejected under 35 U.S.C. § 102(b) as being anticipated by Lock et al. (*Microb. Pathogen.* 21:71-83, 1996). Applicants have amended claim 1 to include the element that the modified pneumolysin comprise at least one amino acid substitution in the region comprising amino acids 1 to 257 and that the polypeptide are refoldable. Therefore, applicants request reconsideration and withdrawal of the Section 102 rejection. Applicants have also amended claims 1 and 2 to recite that the substitution is one besides a substitution of threonine for isoleucine at position 172. This amendment is supported by the specification at the bottom of page 5 which discusses the paper by Lock et al. Through this amendment applicants simply require that the claimed pneumolysins include at least one mutation other than a threonine for isoleucine at position 172, whether or not such a mutation of position 172 is present.

Claims 1 and 22-26 stand rejected under 35 U.S.C. §102(b) as being anticipated by Paton et al. (*Infect. Immun.* 59:2297-2304, 1991). The Examiner contends that Paton et al. describes two pneumolysoids each with a single mutation; Cys428→Gly and Trp433→Phe. Applicants' claim amendments obviate this ground of rejection. Applicants' claims presently describe a pneumolysin which comprises a mutation among amino acids 1-257 of SEQ ID NO: 3. A mutation in this region of pneumolysin is not

disclosed by Paton et al. Therefore, reconsideration and withdrawal of the Section 102 rejection is respectfully requested.

RESPONSE TO OBJECTIONS

Claims 3-6, 13, 23 and 26 stand objected to. Applicants have addressed the Examiner's objections as follows:

(a) Applicants have moved the recitation "SEQ ID NO: 3" from the end of claim 4 to a position following the term "Formula I." Applicants have also inserted the phrase "said Formula I comprising" as suggested by the Examiner.

(b) Commas have been inserted after the term "lysine" in claims 5 and 6.

(c) "Cystine" has been corrected to "cysteine" in claim 6

(d) The term "a" has been deleted from claim 23.

(e) The term "serotypes" has been changed to "types" in claim 26.

(f) The term "pneumococcal" has been changed to "pneumococcus" in claim 23.

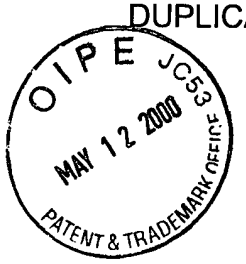
(g) The recitation "Haemophilus influenzae" has been changed to "*Haemophilus influenzae*" in claims 23 and 26.

(h) A period has been inserted at the end of claim 13.

In light of the amendments and arguments presented above, applicants believe the claims are in condition for allowance. Early and favorable response is requested.

AUTHORIZATION

No fee is deemed necessary in connection with the filing of this Communication. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 13-4500, Docket No. 1758-4036US2. A DUPLICATE COPY OF THIS COMMUNICATION IS ATTACHED.



Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on May 9, 2000

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